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I also certify that the attached copy of the request for grant of a Patent (Form 1/77) bears an amendment, effected by this office, following a request by the applicant and agreed to by the Comptroller-General.

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1. Your reference

AGS/P19933GB

2. Patent application number
(The Patent Office will fill in this part)

9624204.5

21 NOV 1996

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

DALGETY PLC
100 George Street
London W1H 5RH
GB

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

PRODUCTION OF VEGETABLE GELS

5. Name of your agent *(if you have one)*

~~KILBURN & STRODE~~

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

~~30 John Street~~
~~London WC1N 2DD~~
~~GB~~

Fry Heath & Spence
The Old College
53 High Street
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Surrey. RH6 7BN

Patents ADP number *(if you know it)*

125001

form 1/77 filed 14.1.97 LB 16197

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Pat nts Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	9
Claim(s)	4
Abstract	–
Drawing(s)	–

10. If you are also filing any of the following, state how many against each item.

Priority documents	–
Translations of priority documents	–
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	X
Request for preliminary examination and search *(Patents Form 9/77)*	X
Request for substantive examination *(Patents Form 10/77)*	–
Any other documents *(please specify)*	–

11. I/We request the grant of a patent on the basis of this application.

Signature Kilburn & Strode Date 21-11-96

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr. A.G. Sheard
0171-242 8291

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PRODUCTION OF VEGETABLE GELS

The present invention relates to hemicellulose-based gels and viscous media, to processes for their production, to products containing such gels and/or viscous media and to various applications thereof. In particular, the present invention relates to an improved method for performing oxidative gelation of hemicelluloses which avoids the need for the addition of hydrogen peroxide.

Plant tissue, especially cell wall material, contains hemicelluloses. Hemicelluloses are complex mixtures of noncellulosic cell wall polysaccharides, including pentosans such as arabinoxylans. Convenient sources of hemicelluloses include cereals (such as maize, barley, wheat, oats, rice), pulses (e.g. soya), legumes and fruit.

There are many known methods for fractionating plant material (such as testaceous or cell wall material) to produce hemicellulose and cellulose fractions. Such methods usually involve alkali or water extraction to yield insoluble cellulose and soluble hemicellulose fractions, followed by separation. The soluble extract is then often neutralized (or acidified) to precipitate hemicelluloses. Organic solvents are also commonly used instead of (or in addition to) acidification to precipitate further hemicellulose fractions.

Aqueous extracts of many hemicellulose fractions are known to form gels (or viscous media) when treated with oxidizing agents. The phenomenon is known as "oxidative gelation" in the art, but the term is used herein in a somewhat broader sense to include the case where viscous solutions are produced rather than true gels. This reflects the fact that oxidative gelation is a progressive phenomenon which may be controlled to vary the degree of gelation to the extent that hard, brittle gels are formed at one extreme and slurries, gravies or viscous liquids at the other.

The biochemical basis of the gelling process is not yet fully understood. However, gel formation and/or viscosity increases are thought to arise (at least in part) from cross linking within and/or between macromolecular components of the hemicellulose mediated by ferulic acid residues (for example, involving diferulate generated by oxidative coupling of the aromatic nucleus of ferulic acid). These ferulic

acid residues occur on arabinoxylans present in the hemicellulose. Extensive hydrolysis (by e.g. harsh alkaline treatments) is known to strip the ferulic acid residues from the bulk pentosans, and so hemicelluloses for use as starting materials in the production of gels or viscous solutions are usually extracted by water (particularly hot water) or mild alkali extraction.

As used herein (and as is usual in the art), the terms "ferulic acid" and "ferulate" are used *sensu lato* encompass feruloyl (often denoted feruloyl) groups (i.e. 4-hydroxy-3-methoxy-cinnamyl groups) and derivatives (particularly oxidized derivatives) thereof.

Only a few oxidizing agents are known to have the ability to induce gelation, and these include hydrogen peroxide (usually in conjunction with a peroxidase), ammonium persulphate and formamidine disulphide.

WO 96/03440 describes the use of an oxidase (preferably a laccase) for promoting oxidative gelation of *inter alia* arabinoxylans. However, laccase may not be acceptable for use in certain food applications, is relatively expensive and the supply is limited. Moreover, oxidases such as laccase are relatively weak oxidation-promoters, and the range of different gel strengths obtainable by the use of such enzymes is limited. Indeed, it is possible that the crosslinking acheived through the use of laccase and other oxidases differs fundamentally from that mediated by e.g. hydrogen peroxide, so that the gels may differ significantly in structure from those produced by other forms of oxidative gelation.

WO 93/10158 describes oxidative gelation of hemicellulosic material using an oxidizing system comprising a peroxide (such as hydrogen peroxide) and an oxygenase (such as a peroxidase). However, hydrogen peroxide is inconvenient as a reagent in industrial-scale processes, and is potentially dangerous.

There is therefore a need for alternative methods of promoting oxidative gelation which avoid the aforementioned problems.

Thus, according to the present invention there is provided a hemicellulosic material comprising an oxidase (e.g. glucose oxidas) and optionally a peroxidas (e.g. horse radish peroxidase) supplement.

Th hemicellulosic material may be obtained by any of the standard techniques known in the art for obtaining hemicelluloses suitable as starting materials for oxidative gelation. Preferably, the hemicelluloses are obtained by any of the processes described in WO 93/10158.

As used herein, the term "supplement" as applied to any specified enzyme activity is intended to embrace not only the case where an appropriate enzyme preparation is added during production, but also encompasses the case where endogenous enzyme activity is activated, enhanced, induced or derepressed by any treatment (e.g. chemical or physical treatment) of the hemicellulosic material. Thus, the hemicellulosic material of the invention exhibit supplemental oxidase (and optionally peroxidase) activity howsoever achieved (so long as the level(s) of enzyme activity are sufficient e.g. to promote oxidative gelation), and are not essentially limited to hemicellulose preparations which have been prepared in any particular way.

Preferably, however, the enzyme supplement is added isolated enzyme having the desired activity. The level of purity and/or specificity is not crucial to the practise of the invention, so long as oxidase and/or peroxidase levels are elevated to levels sufficient to promote oxidative gelation under appropriate conditions.

As used herein, the term peroxidase denotes an enzyme which catalyses the general reaction:

$$H_2O_2 + H_2A \Rightarrow 2H_2O + A$$

where H_2A is any oxidisable substrate. Without wishing to be bound by any theory, it is thought that in the case of oxidative gelation the substrate is the polysaccharide/ferulic acid complex, so resulting in crosslinking between the oxidized ferulic acid components *via* formation of a new C-C bond and the production of diferulic acid.

Preferred according to the present invention is peroxidase EC 1.11.1.7 (e.g. horse radish peroxidase). Alternatively, naturally occuring peroxidase activity endogenous to the hemicellulose material may be exploited according to the invention.

As used herein, the term oxidase denotes an enzyme which catalys s the general reaction:

$$O_2 + AH_2 \Rightarrow H_2O_2 + A$$

Where AH_2 is glucose and the enzyme glucose oxidase, the reaction is:

$$O_2 + H_2O + \text{glucose} \Rightarrow H_2O_2 + \text{gluconic acid}$$

Preferred according to the present invention is glucose oxidase EC 1.1.3.4 (e.g. *A. niger* as source). Other oxidases which are suitable for use in the invention include amino acid oxidases, diamine oxidases and xanthine oxidase.

The gelation system of the invention avoids the dangers associated with excess of hydrogen peroxide (which carries a risk of explosion): in the gels of the invention a "negative feedback" loop ensures that if temperature rises due to excessive hydrogen peroxide production then the enzymes producing the hydrogen peroxide are progressively denatured as the temperature rises, so limiting the production of further hydrogen peroxide.

The gelation system of the invention also has further important and unexpected advantages which *inter alia* permit the formulation of self-gelling powders or solutions.

The material of the invention may further comprising an oxidase substrate (e.g. glucose) supplement. If glucose is used, then this has the ancillary advantage of acting as a dispersant. Alternatively, endogenous substrates naturally present in the hemicellulose may be exploited.

The hemicellulosic material is conveniently derived from cereal husk or bran, or legumes, e.g. from maize, wheat, barley, rice, oats or malt, though any source of hemicellulos may be used in the invention so long as it is subject to at least some degree of oxidative gelation.

Preferably, the hemicellulosic material comprises a pentosan, e.g. a water soluble or alkali soluble pentosan fraction. Particularly preferred are materials wherein the pentosan comprises arabinoxylan, for example arabinoxylan ferulate. In one preferred embodiment, the hemicellulose of the invention consists (or consists essentially) of arabinoxylan ferulate.

The material of the invention preferably takes the form of a powder, for example a substantially anhydrous powder. Powders according to the invention preferably contain a dispersant (e.g. glucose or maltodextrin). Such powders are conveniently formulated so as to be self-gelling on the addition of water in the presence of air, for example being formulated to contain oxidase, oxidase substrate (e.g. glucose) and optionally peroxidase supplements.

The invention also contemplates the material as described herein in the form of an aqueous solution. For some applications, such aqueous solutions are preferably oxygen free and packaged in containers which effectively exclude oxygen. Such solutions may be formulated so as to be is self-gelling on exposure to oxygen (e.g. the oxygen in ambient air), for example being formulated to contain oxidase, oxidase substrate (e.g. glucose) and optionally peroxidase supplements.

Also contemplated by the invention are gels or viscous media comprising the material of the invention which has been oxidatively gelled. Such gels or viscous media may comprise (or consist essentially of) cross linked arabinoxylan.

The invention also contemplates a process for preparing a gel or viscous medium comprising the step of oxidatively gelling the materials of the invention, for example by adding water to the anhydrous self-gelling powders or by exposing the oxygen free solutions to air or oxygen.

In another aspect, the invention contemplates a process for effecting oxidative gelation of a hemicellulosic material comprising the step of promoting the generation of hydrogen peroxide *in situ* by redox enzymes.

The redox enzymes preferably comprise an oxidase (e.g. glucose oxidase) and a peroxidase (e.g. horse radish peroxidase), which are preferably present as supplements in the hemicellulosic material.

According to this aspect of the invention, the process may comprise the steps of supplementing a hemicellulosic material with an oxidase and optionally an oxidase substrate and/or a peroxidase. The generation of hydrogen peroxide is then preferably promoted by:

(a) providing oxygen to the material (e.g. by generating oxygen *in situ*); and/or

(b) providing water to the material; and/or

(c) providing oxidase substrate to the material (e.g. by generating substrate *in situ*); and/or

(d) activating one or more of the redox enzymes (e.g. chemically or physically), wherein the provision of oxygen or substrate may be by controlled release or generation *in situ*, for example triggered generation or release by heat, irradiation or chemical treatment.

Where the oxygen is provided by triggering chemical production *in situ*, the invention finds particular application in retort cooking when the gel can be induced to form only on heating.

The invention also contemplates a gel or viscous medium produced by (or obtainable by) any of the processes of the invention.

In another aspect, the invention contemplates a process for producing a hemicellulosic material comprising the step of supplementing a hemicellulose with an oxidase (e.g. glucose oxidase) and optionally a peroxidase (e.g. horse radish peroxidase), and also contemplates materials produced by (or obtainable by) such a process.

The invention also contemplates a pharmaceutical or cosmetic preparation or medical device comprising the material, gel or viscous medium of the invention. The preparation or device may for example be selected from: a wound plug, wound

dressing, wound debriding system, controlled release device, an encapsulated medicament or drug, a lotion, cream, suppository, pessary, spray, artificial skin, protective membrane, a neutraceutical, prosthetic, orthopaedic, ocular insert, injectant, lubricant or cell implant matrix. In such embodiments the material, gel or viscous medium of the invention may further comprising an antibiotic, electrolyte, cell, tissue, cell extract, pigment, dye, radioisotope, label, imaging agent, enzyme, co-factor, hormone, cytokine, vaccine, growth factor, protein (e.g. a therapeutic protein), allergen, hapten or antigen (for e.g. sensitivity testing), antibody, oil, analgesic and/or antiinflammatory agent (e.g. NSAID).

The invention also covers the material, gel or viscous medium of the invention for use in therapy, surgery, prophylaxis or diagnosis, for example in the treatment of surface (e.g. skin or membrane lesions, e.g. burns, abrasions or ulcers).

In a particularly preferred embodiment, the invention contemplates a wound dressing comprising the material of the invention, for example in the form of a spray. Such wound dressings are particularly useful for the treatment of burns, where their great moisture retaining properties help to prevent the wound drying out. Particularly preferred for such application is the self-gelling liquid of the invention which gels on contact with oxygen in the air. Such compositions can be provided in the form of oxygen-free liquids in airtight containers which can be sprayed onto the skin, whereupon the liquid gels after exposure to the air. Such composition may advantageously be formulated so as to produce a slight excess of hydrogen peroxide on exposure to oxygen, so that a sterilizing, antibacterial, bacteriostatic and/or cleansing effect is obtained which helps promote healing.

The invention also contemplates water absorbant nappies, diapers, incontinence pads, sanitary towels, tampons and panty liners comprising the materials and gels of the invention, as well as domestic and industrial cleaning or liquid (e.g. water) recovery operations (e.g. in the oil industry).

Alternatively, the gels of the invention can be provided in the form of hydrated or dehydrated sheets or pellicles for application to various internal or external surfaces of the body, for example during abdominal surgery to prev nt adhesions.

Other embodiments include enzyme immobilizing systems and brewing adjuncts.

Also contemplated is a bread improver comprising the material, gel or viscous medium of the invention.

The invention also covers a foodstuff, dietary fibre source, food ingredient, additive, lubricant, supplement or food dressing comprising the material, gel or viscous medium of the invention. Such products are preferably selected from crumb, alginate replacer, cottage cheeses, aerosol toppings, frozen yoghurts, milk shakes, ice cream, low calorie products such as dressings and jellies, batters, cake mixes, frozen chips, binders, gravies, pastas, noodles, doughs, pizza toppings, sauces, mayonnaise, jam, preserve, pickles, relish, fruit drinks, syrups, toppings and confectionary (e.g. soft centres), petfood (wherein the gel e.g. acts as a binder), a flavour delivery agent, a canning gel, fat replacer (e.g. comprising macerated gel of any one of claims), a coating, a glaze, a bait, a binder in meat and meat analogue products (for example vegetarian products), a gelatin replacer or dairy product or ingredient (e.g. a yoghurt supplement).

When used as a fat replacer the gel of the invention is preferably macerated to optimize its mouthfeel and fat mimetic properties.

The invention will now be described by reference to the following examples which are purely exemplary and which do not limit the scope of the invention in any way.

Example 1

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.5g of glucose and 20mg each of peroxidase and glucose oxidase (Sigma). The composition gelled at 2% in water within 5 min on shaking in air.

Example 2

0.3g of a composition prepared as described in Example 1 was mixed with 6g of Regent (heat-treated) wheat flour and dispersed as a batter (3g of flour mix in 9g of water). The product becam a solid gel in about 10 min.

Example 3

1g of the flour mix prepared as described in Example 2 was mixed with a further 5g of Regent flour and dispersed as a batter (3g of flour mix in 9g of water). The product became a solid gel in about 30 min.

Example 4

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.25g of glucose and 10mg each of peroxidase and glucose oxidase (Sigma). The composition gelled at 2% in water in 8 min on shaking in air.

Example 5

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.125g of glucose and 5mg each of peroxidase and glucose oxidase (Sigma). The composition gelled at 2% in water in 45 min on shaking in air.

Example 6

1.0g of a maize-derived hemicellulosic powder prepared according to the processes described in WO 93/10158 was mixed with 0.063g of glucose and 2.5mg each of peroxidase and glucose oxidase (Sigma). The composition gelled at 2% in water after 2 hours on shaking in air.

CLAIMS

1. A hemicellulosic material comprising an oxidase (e.g. glucose oxidase) supplement.

2. The material of claim 1 further comprising a peroxidase (e.g. horse radish peroxidase) supplement and/or an oxidase substrate (e.g. glucose) supplement.

3. The material of claim 1 or claim 2 wherein the hemicellulosic material is derived from cereal flour, husk or bran, or from legumes (e.g. from maize, wheat, barley, rice, oats or malt).

4. The material of any one of claims 1-3 wherein the hemicellulosic material comprises a pentosan, e.g. a water soluble or alkali soluble pentosan fraction.

5. The material of claim 4 wherein the pentosan comprises arabinoxylan, for example arabinoxylan ferulate.

6. The material of claim 5 wherein the hemicellulosic material consists (e.g. consists essentially of) arabinoxylan ferulate.

7. The material of any one of the preceding claims in the form of a powder, for example a substantially anhydrous powder and optionally a dispersant (e.g. glucose or maltodextrin).

8. The material of claim 7 which comprises oxidase, oxidase substrate (e.g. glucose) and optionally peroxidase supplements, the material being self-gelling on the addition of water.

9. The material of any one of claims 1-8 in the form of an aqueous solution.

10. The material of claim 9 which is substantially oxygen free.

11. The material of claim 10 which comprises oxidase, oxidase substrate (e.g. glucose) and optionally p roxidase supplements and which is self-gelling on exposure to oxygen.

12. A gel or viscous medium comprising the material of any one of claims 1-11 which has been oxidatively gelled.

13. The gel of claim 12 wherein the material comprises (or consists essentially of) cross linked arabinoxylan.

14. A process for preparing a gel or viscous medium comprising the step of oxidatively gelling the material of any one of claims 1-11, for example by adding water to the material of claim 8 or by exposing the material of any one of claims 9-11.

15. A process for effecting oxidative gelation of a hemicellulosic material comprising the step of promoting the generation of hydrogen peroxide *in situ* by redox enzymes.

16. The process of claim 14 wherein the redox enzymes comprise an oxidase (e.g. glucose oxidase) and a peroxidase (e.g. horse radish peroxidase).

17. The process of claim 15 or 16 wherein the process comprises the steps of supplementing a hemicellulosic material with an oxidase and optionally an oxidase substrate and/or a peroxidase.

18. The process of any one of claims 15-17 wherein the generation of hydrogen peroxide is promoted by:

(a) providing oxygen to the material (e.g. by generation or release *in situ*); and/or

(b) providing water to the material; and/or

(c) providing oxidase substrate to the material (e.g. by generation or release *in situ*);

and/or

(d) activating one or more of the redox enzymes (e.g. chemically or physically),

wherein the provision of oxygen or substrate may be by controlled release or generation *in situ*, for example by triggered generation or release by heat, irradiation or chemical treatment(s).

19. A gel or viscous medium produced by (or obtainable by) the process of any one of claims 14-18.

20. A process for producing a hemicellulosic material (for example a material according to any one of claims 1-11) comprising the step of supplementing a hemicellulose with an oxidase (e.g. glucose oxidase) and optionally a peroxidase (e.g. horse radish peroxidase) supplement.

21. A material produced by (or obtainable by) the process of claim 20.

22. A pharmaceutical or cosmetic preparation or medical device comprising the material, gel or viscous medium of any one of the preceding claims, the preparation or device being for example selected from: a wound plug, wound dressing, controlled release device, an encapsulated medicament or drug, a lotion, cream, suppository, pessary, spray, artificial skin, protective membrane, a neutraceutical, prosthetic, orthopaedic, ocular insert, injectant, lubricant or cell implant matrix, optionally further comprising an antibiotic, analgesic and/or antiinflammatory agent.

23. The material, gel or viscous medium of any one of the preceding claims for use in therapy, prophylaxis or diagnosis, for example in the treatment of skin lesions (e.g. burns, abrasions or ulcers).

24. A wound dressing comprising the material of claim 11, for example in the form of a spray.

25. A bread improver comprising the material, gel or viscous medium of any one of the preceding claims.

26. A foodstuff, dietary fibre source, food ingredient, additive, lubricant, suppl ment or dressing comprising the material of any one of claims , the gel or viscous m dium of any one of claims, for example being selected from a p tfood (wherein the g l e.g.

acts as a binder), a flavour deliv ry agent, a canning gel, fat replacer (e.g. comprising macerated gel of any one of claims), a coating, a glaze, a bait or a gelatin replacer.

27. A masking agent comprising the gel of any one of claims , for example for use in masking semiconductor wafers, etching plates or surfaces to be painted.

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Form 237/77 : 10/12/97

AGENT : FRY HEATH & SPENCE